MUTATION IN DIDINIUM NASUTUM

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THE origin of heritable variations or mutations constitutes one of the most fundamental problems of biology. It has long since been recognized that evolution depends upon such variations, and they have consequently been extensively studied by a considerable number of investigators, *e. g.*, Darwin, DeVries, Batson, Kammerer, Tower, Stockard, MacDougal, Jennings, Morgan, *et al.* These studies have resulted in the accumulation of a mass of facts of great importance, but the nature of the origin of the variations in question is still shrouded in mystery.

In a series of experiments on the effect of conjugation and encystment in *Didinium*, extending from April, 1910, to May, 1914, there suddenly appeared, in the latter part of July, 1912, a marked difference in the rate of fission in the progeny of a single individual. This difference appears to have been permanent, as the results presented herewith indicate. And it seems to show, in opposition to the conclusions reached by a considerable number of investigators, that variations in organisms reproducing asexually are at times heritable.

The difference in rate of fission mentioned was discovered in a group of five pure lines, all of which had been carried from the beginning of the experiment. These lines all originated from the same individual, and before the mutation occurred they had produced, without conjugating, an average of 721 generations; and without encysting, 197 generations. Throughout this entire period there was remarkably little difference in the rate of fission in the five lines. The total number of fissions produced by these lines during the 40 days immediately preceding the appearance of the mutation was respectively 164, 171,

168, 166, and 168. Thus it is obvious that in the ancestors of the mutants nothing in the nature of mutations in the rate of fission had occurred for many generations. This indicates that mutations do not ordinarily occur in asexual reproduction.

During the period of 40 days mentioned above, ending July 10, all of the lines were in excellent condition and not a single individual died. On July 12, however, one line died out and on the 14th three more died, leaving but one line. For several days preceding the temperature was very high. It was recorded twice daily and these records show that it reached a maximum on July 9, when it was 28.5° at 7:30 A.M. and 31° at 6 P.M. It was, however, high continuously from the 4th on, and during this period reproduction was exceedingly rapid, as Table I indicates. It was at the close of this period of rapid multiplication that the four lines mentioned above died out and it is probable that this extraordinary environmental condition had much to do with the nature of the variations in the progeny of the remaining line, although similar variations did not occur in four other groups of lines that were running parallel with the one under consideration.

From the remaining line mentioned above five new lines were started on July 15. For the first five days, the rate of fission in these lines was nearly the same, the total number per line being 26, 27, 28, 26 and 28, respectively. During the next five days the difference became somewhat greater, the total number of fissions per line being 21, 19, 18, 17 and 17, respectively. On the day following this period, the line which had produced 19 fissions died out and was replaced by a new line from the one which had produced 21. There were thus two lines having more rapidly, and three lines having less rapidly, dividing ancestors, all, however, originating from the same individual. On October 14 the lines in both groups were increased to five and thus they were continued until the close of the experiment, which extended through 315 days. The individuals in all of the lines in each group repro-

TABLE I

THE RELATION BETWEEN THE FISSION-RATES OF TWO STRAINS OR GROUPS OF LINES ISOLATED FROM THE PROGENY OF A SINGLE DIDINIUM

June 1, 1912 to May 27, 1913

Each column under the brackets represents a line and each number in the column the total number of fissions for five days; *d* indicates that the line died out; *c*, that it encysted; the brackets show the ancestry of the new lines established. The ancestors of the first five lines had passed through 553 generations without conjugation when these lines were started. Not all the lines that died out are indicated in the table. Whenever a line died out more than once in a five-day period, as sometimes happened, it is recorded only once.

Pure Line with 553 ± Generations without Conjugation								Average Total No. Fissions for Five-day Periods	
								First Group of Lines	Second Group of Lines
23	23				22	21	22		
14	15				14	15	15		
17	18				18	17	17		
18	19				18	18	21		
20	21				21	21	20		
23	22				22	22	21		
21	22				21	21	23		
28	31				32	31	29		
Total 164	171				168	166	168		
30	*d*				*d*	*d*	*d*		
26	27				28	26	28	$26\frac{1}{2}$	$27\frac{1}{3}$
21	19				18	17	17	20	$17\frac{1}{3}$
22	*d*				22	*d*	*d*	22	22
17	18				15	15	15	$17\frac{1}{2}$	15
20	20				15	16	16	20	$15\frac{2}{3}$
d	26				20	22	21	26	21
24	24				17	20	19	24	$18\frac{2}{3}$
25	26				18	24	19	$25\frac{1}{2}$	$20\frac{1}{3}$
20	21				13	18	15	$20\frac{1}{2}$	$15\frac{1}{3}$
c	19				15	16	15	19	$15\frac{1}{3}$
26	25				20	22	21	$25\frac{1}{2}$	21
25	24				19	19	20	$24\frac{1}{2}$	$19\frac{1}{3}$
17	17				14	15	14	17	$14\frac{1}{3}$
15	16				12	12	11	$15\frac{1}{2}$	$11\frac{2}{3}$
d	13				9	9	9	$11\frac{1}{2}$	9
7	7				3	5	3	7	$3\frac{2}{3}$
15	16				12	11	9	$15\frac{1}{2}$	$10\frac{2}{3}$
d	18				13	13	15	18	$13\frac{2}{3}$
8	8	9	10	8	6	7	5	$8\frac{3}{5}$	6
13	12	12	12	*c*	8	9	9	$12\frac{1}{4}$	$8\frac{2}{3}$

TABLE I (*continued*)

Pure Line with 553 ± Generations without Conjugation										Average Total No. Fissions for Five-day Periods	
										First Group of Lines	Second Group of Lines
d	10	11	9	*c*	8		8		7	10	$7\frac{2}{3}$
c	12	12	12	12	9		9		*d*	12	9
12	12	13	13	13	*d*	8	8	9	9	$12\frac{3}{5}$	$8\frac{1}{2}$
12	11	11	10	11	9	8	8	8	7	11	8
9	10	10	10	10	*d*	5	6	7	6	$9\frac{4}{5}$	6
9	10	10	*d*	11	7	5	6	6	*d*	10	6
10	10	*d*	8	9	5	3	5	*d*	5	$9\frac{1}{4}$	$4\frac{1}{2}$
9	9	9	9	9	5	*d*	5	*d*	6	9	$5\frac{1}{3}$
10	10	10	*d*	8	7	5	7	6	6	$9\frac{1}{2}$	$6\frac{1}{5}$
10	*c*	*d*	*d*	9	6	7	7	7	7	$9\frac{1}{2}$	$6\frac{4}{5}$
10	11	12	11	10	8	9	8	8	8	$10\frac{4}{5}$	$8\frac{1}{5}$
11	*c*	13	*c*	13	*c*	7	8	*d*	5	$12\frac{1}{3}$	$6\frac{2}{3}$
11	12	12	12	12	8	*c*	6	8	4	$11\frac{4}{5}$	$6\frac{1}{2}$
d	13	*d*	13	12	9	6	9	8	8	$12\frac{2}{3}$	8
d	14	14	14	14	10	12	10	10	10	14	$10\frac{2}{5}$
15	*d*	13	14	14	10	10	9	10	10	14	$9\frac{4}{5}$
12	12	12	13	12	9	8	8	8	8	$12\frac{1}{5}$	$8\frac{1}{5}$
13	13	13	15	14	10	10	9	10	10	$13\frac{3}{4}$	$9\frac{4}{5}$
12	11	11	*c*	12	9	9	8	9	8	$11\frac{1}{2}$	$8\frac{3}{5}$
10	10	10	11	10	8	9	7	8	6	$10\frac{1}{5}$	$7\frac{3}{5}$
11	11	11	11	11	8	8	8	8	7	11	$7\frac{4}{5}$
10	10	11	10	10	5	7	6	6	*d*	$10\frac{1}{5}$	6
8	11	11	11	10	8	8	6	7	8	$10\frac{1}{5}$	$7\frac{2}{5}$
10	13	12	11	12	9	11	8	8	8	$11\frac{4}{5}$	$8\frac{1}{5}$
11	14	*d*	*d*	*d*	10	6	11	11	10	$12\frac{1}{2}$	$9\frac{3}{5}$
10	10	*d*	12	9	9	7	*d*	8	*d*	$10\frac{1}{4}$	8
7	8	9	9	8	7	7	7	7	7	$8\frac{1}{5}$	7
9	13	12	12	*d*	10	8	9	9	9	$11\frac{1}{2}$	9
7	12	13	13	11	10	8	8	9	8	$11\frac{1}{5}$	$8\frac{3}{5}$
10	14	12	12	14	9	8	9	9	10	$12\frac{2}{5}$	9
8	12	12	11	12	9	7	8	9	7	11	8
d	9	8	9	9	6	*d*	*d*	6	6	$8\frac{3}{4}$	6
10	11	*d*	11	11	9	7	6	*d*	6	$10\frac{1}{2}$	7
8	8	8	6	*d*	3	7	7	6	6	$7\frac{1}{4}$	$5\frac{4}{5}$
9	9	7	*d*	*d*	7	*d*	7	7	7	$8\frac{1}{3}$	7

TABLE I (*concluded*)

Pure Line with 553 ± Generations without Conjugation										Average Total No. Fissions for Five-day Periods	
										First Group of Lines	Second Group of Lines
9	9	8	7	7	6	6	7	5	5	8	$5\frac{4}{5}$
12	*d*	11	12	13	9	9	8	*d*	7	12	$8\frac{1}{4}$
7	9	10	9	9	6	5	3	*d*	7	$8\frac{4}{5}$	$5\frac{1}{4}$
16	11	13	14	14	*d*	10	7	*d*	9	$13\frac{3}{5}$	$8\frac{2}{5}$
d	7	7	8	6	4	3	5	4	4	7	4
10	9	9	9	6	7	3	4	4	4	$8\frac{3}{5}$	$4\frac{2}{5}$
10	8	11	7	9	8	7	8	7	*d*	9	$7\frac{1}{2}$
d	8	10	*d*	10	6	*d*	8	5	*d*	$9\frac{1}{3}$	$6\frac{1}{3}$
Total average generations per line in 315 days										838 ±	634 ±
Number of lines died out										30	33
Number of lines encysted										8	3
Number of times conjugation occurred before transfer was made										2	0

duced at practically the same rate, but those in the former, considerably more rapidly than those in the latter. During the 315 days each line in the one group produced approximately 838 generations, $2\frac{2}{3}$ per day, and each line in the other group approximately 634 generations, 2 per day.

The averages for the five lines in each group for five-day periods are presented in Table I and plotted in Fig. 1. By referring to this table and the figure it will be seen that the difference in rate of fission in the two groups remained fairly constant throughout the entire 315 days, and that in both groups the rate was high in July and August, 1912, after which it decreased considerably and then remained fairly constant.

The fluctuations in rate of fission were closely associated with variations in temperature. This was true for twenty-four-hour periods as well as for the five-day periods given in the table. During July and August, when the fission rate was high, the temperature was in general much higher than it was during the rest of the time, when the fission rate was relatively low. At the

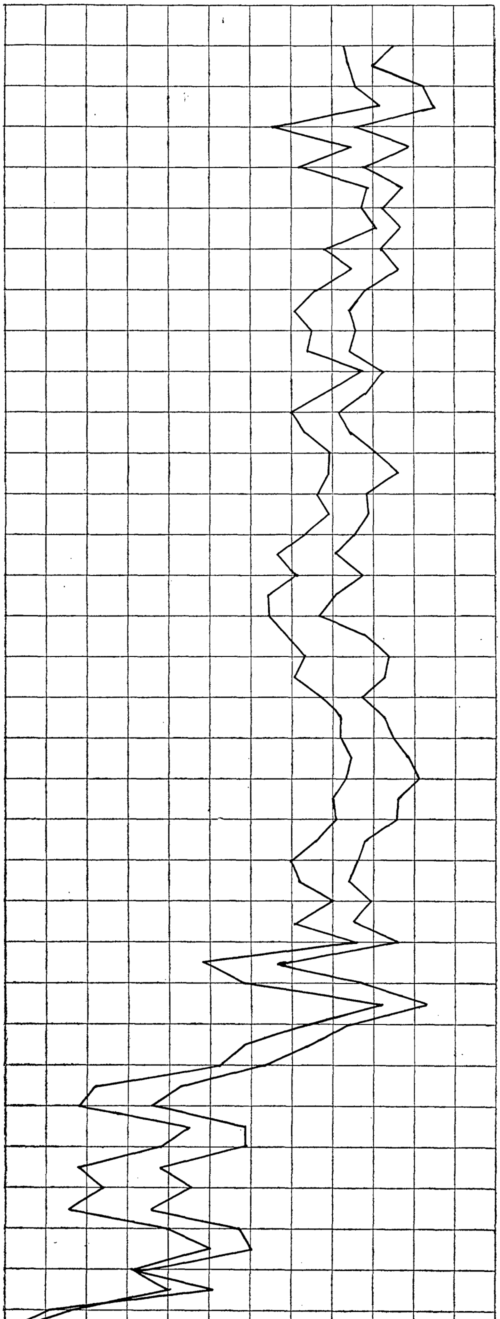

FIG. 1. Curves representing the fission rates in two groups of lines of *Didinium* originating from a single individual 721 ± generations after conjugation and 197 ± generations after encystment. Each point in the curves was obtained by averaging the rate of fission of all of the lines in each group for five successive days. Ordinates equal average daily rate of fission, abscissas days from the beginning of the experiment.

Note that the difference in the rate of fission in the two groups is essentially the same during the entire 315 days.

close of the experiment, however, the rate of fission was not as high as the temperature at this time would lead one to expect. The didinia in both groups appeared to be in poor condition. There were numerous very small individuals produced and an unusually large number of monsters. Conjugation was prevalent, but it was almost impossible to induce encystment. The death-rate was, however, not abnormally high. Whether or not the lines would have recovered from this depression if the experiment had been continued, is a question which can not be definitely answered.

Before the experiment was closed some cysts were secured in both groups. These were kept in a damp chamber as usual until the following year. Then they were put into culture fluids of various sorts containing paramecia; but only a few developed, all of which belonged to the more rapidly dividing lines. From these, five new lines were started and carried on for 40 days. During all this time the condition of the individuals was much as it had been immediately before encystment.

Throughout the entire experiment the didinia were cultivated in rectangular watch-glasses having a depression with a curved bottom. These dishes were piled one upon the other and kept in a damp chamber. All of the didinia were fed with paramecia from the same cultures. At each feeding an equal amount of solution was taken from two of the most vigorous of four pint cultures which were continuously kept in as flourishing conditions as possible by adding fresh water and a little timothy hay from time to time. The two equal quantities of solution were then thoroughly mixed and two drops of this mixture containing numerous paramecia were put into each of as many watch-glasses as there were didinia cultures. One drop of solution containing one didinium was then taken from each of the didinia cultures and added to each of the watch-glasses containing the paramecia. The remaining didinia, after recording the number of generations produced, were destroyed or used in studying conjugation

and encystment. During the coldest weather it was sufficient to transfer every other day, but during the warmest weather it was found advantageous to transfer twice a day. Nothing was sterilized in these experiments, but the same pipet was used in all transfers and the watch-glasses not in use were exposed to the air and allowed to dry. Moreover, from time to time the didinia in each line in either group were transferred directly to the watch-glasses from which the didinia of the other group had just been taken. In these dishes there always remained considerable solution, in some instances a drop or more. Furthermore, in a few cases didinia from the more rapidly dividing lines were transferred directly without the addition of fresh food to dishes in which more slowly dividing lines had died or from which all of the didinia had been removed.

Such treatment had no appreciable effect on the relative rate of fission in the two races. It is obviously evident, therefore, that the difference in the rate observed was not due to difference in the bacterial contents of the solution if there really was any such difference, nor was it dependent upon selection, natural or otherwise, for members of the more rapidly dividing pairs were always transferred in all lines. And the number of lines lost by death and encystment was essentially the same in both. In the one 30 were lost by death and 8 by encystment, in the other 33 by death and 3 by encystment. Assuming that the weaker lines died out in every case, it is evident that in this respect both races were subjected to practically the same sort of selection. And since all of the cultures were subjected to the same conditions otherwise, it is clear that the difference in the rate of fission in the two races must have been due to the constitution of the organisms.

We have consequently demonstrated that marked variations in the rate of fission may appear quite suddenly in the progeny of a single individual without conjugation or encystment, that some of these variations are heritable, and that they can probably be produced by subjecting the individuals to abnormally high temperature.

By referring to the table it will be seen that the mutation investigated originated, as previously stated, at the close of a period of extraordinarily high rate of fission and immediately after a short period of very high death-rate in which all but one of the lines died out. At the beginning of this period and at the close of the preceding period the individuals were very small and showed all the characteristics in behavior common to individuals about to conjugate. Whether or not anything in the nature of a nuclear reorganization in preparation for conjugation occurred in these didinia is not known, but ordinarily such phenomena do not begin until some time after union takes place in conjugating specimens. Moreover, the period between fissions was not long enough to admit of much in the way of reorganization aside from what ordinarily occurs during the process of fission. Whether or not the ancestors of the mutants were actually homozygous is not known. If they were not the mutation may possibly have been due to a rearrangement of unit characters represented in the chromosomes during fission resulting in a change in dominance. However, if this did actually take place it is not in accord with the results obtained in very extensive investigations, all of which seem to show that changes in dominance do not occur in asexual reproduction. It is probable, therefore, that the mutation was due to a direct effect of the environment on the physiological processes in the organism and not to inherited nuclear phenomena largely independent of the immediate environment.

The mutation theory so ably championed by DeVries has of late lost greatly in prestige, owing largely to the contention that the plants (*Œnothera*) in which DeVries discovered mutations were hybrids. If the conclusion reached in this work proves to be correct it will strongly support the theory in question. It will demonstrate that marked variations may appear suddenly in organisms reproducing asexually, that such variations may be heritable and that they may have a decided evolutionary value.

This conclusion, though in opposition to a great bulk of the experimental evidence gathered by some of the foremost biologists, Johannsen, Maupas, Morgan, Castle, Jennings and many others, is supported by some of the results obtained by Barber (1907), Calkins and Gregory (1913), Middleton (1915) and Jennings (1916).

SUMMARY

In a race of didinia originating from a single individual there suddenly appeared a heritable variation in the rate of fission. This variation occurred 721 generations after conjugation and 197 generations after encystment.

Two strains were isolated from this race and kept under observation for 315 days. During this time the lines in one strain produced an average of 838 $\pm$ generations ($2\frac{2}{3}$ per day) and those of the other 634 $\pm$ generations (2 per day).